
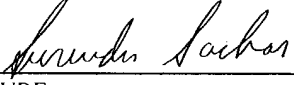


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|---|--|---|--|
| FORM PTO-1390 (Modified)<br>(REV 11-2000)   |  | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER<br><b>217365US0PCT</b>                  |
| TRANSMITTAL LETTER TO THE UNITED STATES<br>DESIGNATED/ELECTED OFFICE (DO/EO/US)<br>CONCERNING A FILING UNDER 35 U.S.C. 371  |  |   | U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR<br><b>09/926778</b> ) |
| INTERNATIONAL APPLICATION NO.<br><b>PCT/FR00/01670</b>  | INTERNATIONAL FILING DATE<br><b>16 June 2000</b> | PRIORITY DATE CLAIMED<br><b>18 June 1999</b>            |  |
| TITLE OF INVENTION<br><b>COMPOSITIONS CONTAINING SOLUBLE FORMS OF HLA-G IN THE TREATMENT OF INFLAMMATORY SKIN PATHOLOGIES</b>   |  |   |  |
| APPLICANT(S) FOR DO/EO/US<br><b>ARACTINGI Selim et al.</b>  |  |   |  |
| Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:   |  |   |  |
| <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> <li>11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li>12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> </ol> |  |   |  |
| <b>Items 13 to 20 below concern document(s) or information included:</b>  |  |   |  |
| <ol style="list-style-type: none"> <li>13. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>15. <input type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>16. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>17. <input type="checkbox"/> A substitute specification.</li> <li>18. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</li> <li>20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>22. <input type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>23. <input checked="" type="checkbox"/> Other items or information: <p><b>Request for Priority/Drawings (4 sheets)</b><br/><b>Form PTO-1449</b><br/><b>Sequence Listing (1 page)</b></p> </li> </ol>  |  |   |  |

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| U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR<br><b>09/926778</b>   |              | INTERNATIONAL APPLICATION NO.<br><b>PCT/FR00/01670</b> |           | ATTORNEY'S DOCKET NUMBER<br><b>217365US0PCT</b> |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
|--|--------------|--|-----------|---|--------------|--------------|------|--------------|--------|---|-----------|--------------------|-------|---|-----------|---|--|--|--|-----------------|--|
| 24. The following fees are submitted:<br><b>BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5) ) :</b><br><div style="margin-left: 20px;"><input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$1040.00</b><br/><input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$890.00</b><br/><input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$740.00</b><br/><input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$710.00</b><br/><input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b></div> <div style="text-align: right; margin-right: 50px;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div> |              |  |           | <b>CALCULATIONS PTO USE ONLY</b>                |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).   |              |  |           | <b>\$890.00</b>                                 |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <table border="1" style="width:100%; border-collapse: collapse;"><thead><tr><th style="width:15%;">CLAIMS</th><th style="width:20%;">NUMBER FILED</th><th style="width:20%;">NUMBER EXTRA</th><th style="width:15%;">RATE</th></tr></thead><tbody><tr><td>Total claims</td><td style="text-align: center;">- 20 =</td><td style="text-align: center;">0</td><td>x \$18.00</td></tr><tr><td>Independent claims</td><td style="text-align: center;">- 3 =</td><td style="text-align: center;">0</td><td>x \$84.00</td></tr><tr><td colspan="4">Multiple Dependent Claims (check if applicable). <input type="checkbox"/></td></tr></tbody></table>   |              |  |           | CLAIMS  | NUMBER FILED | NUMBER EXTRA | RATE | Total claims | - 20 = | 0 | x \$18.00 | Independent claims | - 3 = | 0 | x \$84.00 | Multiple Dependent Claims (check if applicable). <input type="checkbox"/> |  |  |  | <b>\$130.00</b> |  |
| CLAIMS   | NUMBER FILED | NUMBER EXTRA   | RATE      |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Total claims   | - 20 =       | 0  | x \$18.00 |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Independent claims   | - 3 =        | 0  | x \$84.00 |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Multiple Dependent Claims (check if applicable). <input type="checkbox"/>  |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <b>TOTAL OF ABOVE CALCULATIONS =</b>   |              |  |           | <b>\$1,020.00</b>                               |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.  |              |  |           | <b>\$0.00</b>                                   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <b>SUBTOTAL =</b>  |              |  |           | <b>\$1,020.00</b>                               |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).   |              |  |           | <b>\$0.00</b>                                   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <b>TOTAL NATIONAL FEE =</b>  |              |  |           | <b>\$1,020.00</b>                               |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>   |              |  |           | <b>\$0.00</b>                                   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <b>TOTAL FEES ENCLOSED =</b>   |              |  |           | <b>\$1,020.00</b>                               |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
|  |              |  |           | Amount to be:                                   | \$           |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
|  |              |  |           | refunded  |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
|  |              |  |           | charged   | \$           |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
|  |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <div style="margin-left: 20px;">a. <input checked="" type="checkbox"/> A check in the amount of <b>\$1,020.00</b> to cover the above fees is enclosed.<br/>b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.<br/>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <b>15-0030</b> A duplicate copy of this sheet is enclosed.<br/>d. <input type="checkbox"/> Fees are to be charged to a credit card. <b>WARNING:</b> Information on this form may become public. <b>Credit card information should not be included on this form.</b> Provide credit card information and authorization on PTO-2038.</div>  |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>   |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| SEND ALL CORRESPONDENCE TO:  |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <div style="border: 1px solid black; padding: 10px; margin: 10px 0;"><div style="text-align: center;"><b>Surinder Sachar</b><br/>Registration No. 34,423</div><div style="text-align: center;"><br/><b>22850</b></div></div>  |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |
| <div style="margin-left: 500px;"><div style="text-align: center;"><br/>SIGNATURE</div><div style="text-align: center;"><b>Norman F. Oblon</b><br/>NAME</div><div style="text-align: center;"><b>24,618</b><br/>REGISTRATION NUMBER</div><div style="text-align: center;"><b>Dec. 18 2001</b><br/>DATE</div></div>   |              |  |           |   |              |              |      |              |        |   |           |                    |       |   |           |   |  |  |  |                 |  |

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10 Rec'd PCT/PTO 01 JUL 2002 AS

217365US-0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :  
SELIM ARACTINGI ET AL : ATTN: APPLICATION DIVISION  
SERIAL NO: 09/926,778 :  
FILED: DECEMBER 18, 2001 :  
FOR: COMPOSITIONS CONTAINING :  
SOLUBLE FORMS OF HLA-G FOR TREATING  
INFLAMMATORY SKIN PATHOLOGIES

PRELIMINARY AMENDMENT AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Responsive to the Notice dated March 29, 2002, Applicants submit herewith an  
Amendment, a substitute Sequence Listing, and a corresponding Computer Readable  
Sequence Listing.

IN THE SPECIFICATION

After page 24 (Abstract), beginning on a new page, please delete the original  
Sequence Listing at page 1 and insert the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as shown in the marked-up copy following this amendment to read as follows.

--3. (Amended) The use as claimed in claim 1, characterized in that said composition comprises between 0.1 and 5  $\mu\text{g/ml}$ , preferably between 0.5 and 2.5  $\mu\text{g/ml}$ , of soluble form of HLA-G.--

REMARKS

Claims 1- 7 are active in the present application. Claim 3 has been amended for clarity and to remove multiple dependencies. No new matter is added by the amendment to Claim 3.

In addition, the specification has been amended to insert a substitute Sequence Listing, and a Computer Readable Sequence Listing (CRF) is attached herewith. The contents of the paper copy of the substitute Sequence Listing and the contents of the (CRF) submitted herewith are identical. Support for all the sequences listed in the substitute Sequence Listing can be found in the present application as originally filed. No new matter is introduced by the submission of the substitute Sequence Listing.

Applicants submit that the present application is now in condition for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.



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217365US-0PCT

**Marked-Up Copy**  
Serial No: 09/926,778  
Amendment Filed on:  
HEREWITH

IN THE SPECIFICATION

After page 24 (Abstract), beginning on a new page, please delete the original Sequence Listing at page 1 and insert the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as follows.

--3. (Amended) The use as claimed in claim 1 [or claim 2], characterized in that said composition comprises between 0.1 and 5 µg/ml, preferably between 0.5 and 2.5 µg/ml, of soluble form of HLA-G.--

COMPOSITIONS CONTAINING SOLUBLE FORMS OF HLA-G IN THE  
TREATMENT OF INFLAMMATORY SKIN PATHOLOGIES

5 The present invention relates to the use of compositions containing soluble forms of HLA-G in the treatment of skin pathologies, and in particular of inflammatory dermatoses, to the method for obtaining said soluble forms of HLA-G and also to the antibodies directed against said soluble forms.

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The antigens of the major histocompatibility complex (MHC) are divided up into several classes, the class I antigens (HLA-A, HLA-B and HLA-C) which have 3 globular domains ( $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$ ), the  $\alpha 3$  domain of which is associated with  $\beta 2$  microglobulin, the class II antigens (HLA-DP, HLA-DQ and HLA-DR) and the class III antigens (complement).

20 The class I antigens comprise, besides the abovementioned antigens, other antigens, termed unconventional class I antigens, and in particular the HLA-E, HLA-F and HLA-G antigens; the latter, in particular is expressed by extravillous trophoblasts of normal human placenta and thymic epithelial cells.

25

The sequence of the HLA-G gene (HLA-6.0 gene) has been described by GERAGHTY et al., (Proc. Natl. Acad. Sci. USA, 1987, 84, 9145-9149); it comprises 4396 base pairs and has an intron/exon organization homologous to that of the HLA-A, -B and -C genes. More precisely, this gene comprises 8 exons, 7 introns and a 3' untranslated end; the 8 exons correspond, respectively, to: exon 1: signal sequence, exon 2:  $\alpha 1$  extracellular domain, exon 3:  $\alpha 2$  extracellular domain, exon 4:  $\alpha 3$  extracellular domain, exon 5: transmembrane region, exon 6: cytoplasmic domain I, exon 7: cytoplasmic domain II (untranslated), exon 8: cytoplasmic domain III (untranslated) and 3' untranslated region (GERAGHTY et

30

35

al., mentioned above; ELLIS et al., J. Immunol., 1990, 144, 731-735; KIRSZENBAUM M. et al., *Oncogeny of hematopoiesis, Aplastic anemia* Eds. E. Gluckman, L. Coulombel, Colloque INSERM/John Libbey Eurotext Ltd).

5 However, the HLA-G gene differs from the other class I genes in that the in-frame translation termination codon is located at the second codon of exon 6; consequently the cytoplasmic region of the protein encoded by this HLA-6.0 gene is considerably shorter

10 than that of the cytoplasmic regions of the HLA-A, -B and -C proteins.

These HLA-G antigens are essentially expressed by the cytotrophoblastic cells of the placenta and are

15 considered to play a role in protecting the fetus (lack of rejection by the mother). In addition, in so far as the HLA-G antigen is monomorphic, it may also be involved in the growth or the function of placental cells (KOVATS et al., Science, 1990, 248, 220-223).

20 Other investigations concerning this unconventional class I antigen (ISHITANI et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 3947-3951) have shown that the primary transcript of the HLA-G gene may be spliced in

25 several ways and produces at least 3 distinct mature MRNAs: the primary HLA-G transcript provides a complete 1200 bp copy (G1), a 900 bp fragment (G2) and a 600 bp fragment (G3).

30 The G1 transcript does not comprise exon 7 and corresponds to the sequence described by ELLIS et al. (mentioned above), i.e. it encodes a protein which comprises a leader sequence, three external domains, a transmembrane region and a cytoplasmic sequence. G2

35 mRNA does not comprise exon 3, i.e. it encodes a protein in which the  $\alpha 1$  and  $\alpha 3$  domains are directly joined; the G3 mRNA does not contain exon 3 or exon 4, i.e. it encodes a protein in which the  $\alpha 1$  domain and the transmembrane sequence are directly joined.



The splicing which prevails in order to produce the HLA-G2 antigen leads to the joining of an adenine (A) (originating from the domain encoding  $\alpha 1$ ) with an AC sequence (derived from the domain encoding  $\alpha 3$ ), which leads to the creation of an AAC codon (asparagine) in place of the GAC codon (aspartic acid) encountered at the beginning of the sequence encoding the  $\alpha 3$  domain in HLA-G1.

10

The splicing generated in order to produce HLA-G3 does not lead to the formation of a new codon in the splicing zone.

15 The authors of that article also analyze the various proteins expressed: the 3 mRNAs are translated into protein in the .221-G cell line.

20 Some of the inventors have shown the existence of other spliced forms of HLA-G mRNA: the HLA-G4 transcript, which does not include exon 4; the HLA-G5 transcript, which includes intron 4, between exons 4 and 5, thus causing a modification of the reading frame when this transcript is translated and, in particular, the appearance of a stop codon after amino acid 21 of intron 4; and the HLA-G6 transcript, which contains intron 4 but which has lost exon 3 (KIRSZENBAUM M. et al., *Proc. Natl. Acad. Sci. USA*, 1994, 91, 4209-4213; European Application EP 0 677 582; KIRSZENBAUM M. et al., *Human Immunol.*, 1995, 43, 237-241; MOREAU P. et al., *Human Immunol.* 1995, 43, 231-236). They have also shown that these various transcripts are expressed in several human fetal and adult cell types, in particular in lymphocytes (KIRSZENBAUM M. et al., *Human Immunol.*, 1995, mentioned above; MOREAU P. et al., *Human Immunol.* 1995, mentioned above).

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There are therefore at least 6 different HLA-G mRNAs which potentially encode 6 protein isoforms of HLA-G, 4

of which are membrane-bound (HLA-G1, G2, G3 and G4) and 2 of which are soluble (G5, G6).

5 It has been shown that the expression of said protein isoforms of HLA-G is increased by  $\gamma$ -interferon (Yang et al., J. Immunol., 1996, **156**, 4224-4231).

10 The immunomodulatory function exercised by these HLA-G molecules has been described and the mechanisms of this function have been elucidated by demonstrating their interaction with lysis-inhibiting receptors present on NK cells (KIR receptors), leading to inhibition of the cytotoxic functions of these cells; for example, N. ROUAS-FREISS ET AL. (Proc. Nat. Acad. Sci., 1997, **94**, 15 5249-5254) have shown that K562 (human erythroleukemia line) target cells transfected with the HLA-G gene are protected against lysis by NK cells. These cells are normally sensitive to NK cells.

20 Some of the inventors have shown that NK cells do not express any HLA-G transcript (Teyssier et al., Nat. Immunol., 1995, **14**, 262-270), this result confirming that the products of expression of the HLA-G gene probably play a role in states of physiological 25 immunotolerance (pregnancy) or pathological immunotolerance in which NK cells are particularly active (autoimmune diseases, transplants) or, on the contrary, are inhibited (abnormal presence of HLA-G on certain tumors or during viral infections).

30 Thus, some of the inventors have also shown that certain tumors express HLA-G molecules, which enables them to evade immune surveillance (Paul et al., Proc. Nat. Acad. Sci., 1998, **95**, 4510-4515).

35 Psoriasis is a common chronic inflammatory pathology, and is observed in 2% of individuals in Caucasian populations. Although the disease is characterized by hyperproliferation of the keratinocytes of the

epidermis, a large number of physiopathological studies have made it possible to show that T lymphocytes of the Th1 subtype which infiltrate the site of the lesions and produce interleukin 2 (IL-2) and  $\gamma$ -interferon ( $\gamma$  IFN), play a predominant role in the pathogenesis of this disease (Z. Bata-Csorgo et al., J. Investigative Dermatol., 1995, 89S-94S; Schlaak et al., J. Invest. Dermatol., 1994, **102**, 145-149; Vogel et al., Eur. J. Biochem., 1995, **227**, 143-149). It has been shown that the supernatant of T-cell clones derived from psoriasis lesions is capable of inducing hyperproliferation of the epidermis (Strange et al., J. Invest. Dermatol., 1993, **101**, 695-700). Similarly, it has been shown that maintenance of the psoriatic phenotype of human skin biopsies transplanted into SCID mice is dependent on the coinjection of T lymphocytes derived from said lesions (Gilbar et al., J. Invest. Dermatol. 1997, **109**, 283-288).

The expression of HLA-G in skin cells and its potential roll in the associated pathologies has been studied by Ulbrecht et al. (Eur. J. Immunol., 1994, **24**, 176-180). The authors of that article, who analyze only the expression of the transcripts of the membrane-bound isoforms of HLA-G, and in particular of the dominant isoform HLA-G1, show that, while a low level of HLA-G transcripts is detected in skin biopsies exhibiting psoriasis lesions, HLA-G transcripts are either absent or present at a high level in healthy skin biopsies derived from different individuals. These authors therefore conclude that there is no obvious link between the expression of HLA-G and skin pathologies.

The inventors have now found that soluble forms of HLA-G have a therapeutic action on inflammatory pathological skin conditions.

A subject of the present invention is the use of a composition essentially consisting of at least one

soluble form of HLA-G and of at least one pharmaceutically acceptable vehicle (excipient), for preparing a medicinal product for treating inflammatory pathological skin conditions.

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The excipients combined with said composition are suitable for the desired route of administration; they are selected from excipients known to those skilled in the art.

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Said soluble HLA-Gs may advantageously be administered generally (orally, parenterally) or locally (topical administration).

15 In the latter case, said composition is in the form of cream, of lotion, of liposomes or of gel.

The inventors have now found, unexpectedly, the presence, in inflammatory skin lesions, both of  
20 macrophages expressing the HLA-G protein, which are located in the dermal papillae, and of infiltrating CD3<sup>+</sup> T lymphocytes expressing a receptor for inhibiting cytotoxic functions which is recognized by HLA-G, such as for example the ILT2 receptor.

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The inventors have shown that the dominant membrane-bound isoform HLA-G1 and the soluble isoform HLA-G5 are expressed only in inflammatory skin lesions, whereas no HLA-G protein is detected in healthy skin.

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The inventors have also shown that the HLA-G protein, and in particular an isoform comprising at least the  $\alpha 1$  domain of HLA-G, is capable of inhibiting the proliferative functions and the cytotoxic functions of  
35 T lymphocytes.

Thus, the inventors have therefore demonstrated the anti-inflammatory role of the HLA-G protein, and in particular of a composition consisting of a diffusible

soluble form comprising at least the  $\alpha 1$  domain of HLA-G, such as for example the HLA-G5 isoform, in the treatment of inflammatory skin pathologies.

- 5 In accordance with the invention, said composition is particularly suitable for treating psoriasis.

According to an advantageous embodiment of the invention, said soluble form of HLA-G is selected from  
10 the group consisting of the soluble isoforms of HLA-G comprising at least one extracellular domain ( $\alpha 1$ ,  $\alpha 2$  or  $\alpha 3$ ) and the solubilized forms of HLA-G1, HLA-G2, HLA-G3 or HLA-G4 (membrane-bound isoforms).

- 15 Said soluble HLA-Gs comprise at least the  $\alpha 1$  extracellular domain.

Said soluble forms are in particular produced in a *baculovirus*.

20

With regard to the membrane-bound forms, they are advantageously expressed in eukaryotic cells, in accordance with the method described in International Application PCT WO 98/37098, and then solubilized by  
25 treatment of the membrane (stripping agent, such as papain) and suitable purification, for example on an immunoaffinity column with specific antibodies.

The term "soluble form of HLA-G" is intended to mean  
30 both the soluble HLA-Gs (not comprising a transmembrane domain) and the membrane-bound HLA-Gs which have been solubilized, for example under the conditions specified above.

- 35 Preferably, said composition, administered topically, comprises between 0.1 and 5  $\mu\text{g/ml}$ , preferably between 0.5 and 2.5  $\mu\text{g/ml}$ , of soluble form of HLA-G.

A subject of the present invention is also a method for preparing a soluble HLA-G, characterized in that it comprises the following steps:

- 5    -    coinfecting insect cells with a baculovirus containing the  $\beta_2M$  cDNA and another baculovirus containing the  $\alpha$  chain of a soluble isoform of HLA-G;
- culturing the transfected insect cells, and
- 10   -    harvesting the supernatants and purifying the soluble isoform of HLA-G expressed.

According to an advantageous embodiment of said method,  
15   said soluble isoform of HLA-G is purified using an antibody specific for the soluble isoforms of HLA-G.

According to an advantageous arrangement of this embodiment, said antibody is obtained by immunizing  
20   nonhuman mammals, such as rabbits, with an immunogenic peptide consisting of a 21 amino acid synthetic peptide corresponding to the C-terminal portion encoded by intron 4 of the soluble HLA-G forms, the sequence of which is SKEGDGGIMSVRESRSLSEDL (SEQ ID NO:1), coupled  
25   to the KLH carrier protein.

Besides the above arrangements, the invention also comprises other arrangements, which will emerge from the following description which refers to examples of  
30   implementation of the method which is the subject of the present invention and also to the attached drawings, in which:

- figures 1 and 2 illustrate the presence of the  
35   RNAs of the various isoforms of HLA-G in psoriasis lesions, compared with healthy skin.

- figure 3 illustrates the presence of the RNA specific for the HLA-G5 isoform in psoriasis lesions, compared with healthy skin.
- 5 - figure 4 illustrates the inhibitory activity of the HLA-G isoforms on *natural killer* cells (NK cells) present in peripheral blood; this figure comprises, on the x-axis, the isoform studied and, on the y-axis, the percentage specific lysis. M8 cells (HLA class I<sup>+</sup>,  
10 class II<sup>-</sup> melanoma line cells) transfected either with the vector alone (M8-pCDNA) (Invitrogen) or with the vectors containing the cDNA encoding HLA-G1 (M8-HLA-G1), the cDNA encoding HLA-G2 (M8-HLA-G2), the cDNA encoding HLA-G3 (M8-HLA-G3) or the cDNA encoding HLA-G4  
15 (M8-HLA-G4) are used as targets (T). Peripheral blood mononuclear cells, PBMCs, are used as effector cells (E). The results are expressed as percentage lysis, recorded in 4 h in a chromium 51(<sup>51</sup>Cr)-release assay.
- 20 - figure 5 illustrates the inhibitory activity of the HLA-G isoforms on a CD8<sup>+</sup> T lymphocyte line restricted for HLA-A2 presenting a viral peptide originating from the matrix of the influenza virus, positions 58 to 66; this figure comprises, on the x-axis, the isoform  
25 studied and on the y-axis, the percentage specific lysis; the effector cell/target cell ratio is 15:1.

It should be clearly understood, however, that these examples are given only by way of illustration of the  
30 subject of the invention, for which they in no way constitute a limitation.

**EXAMPLE 1 : Production of a soluble form of an isoform of HLA-G in a baculovirus**

35

The soluble HLA-G5 isoform, the structure of which consists of 3 extracellular domains,  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$ , associated with  $\beta 2$ -microglobulin ( $\beta 2M$ ), is derived from an alternative transcript which has a stop codon in

intron 4 of the HLA-G gene. The production of a recombinant soluble HLA-G protein makes use of coinfection of the insect cells with a baculovirus containing the complementary DNA of  $\beta 2M$ , and another  
5 baculovirus containing the cDNA of the  $\alpha$  chain of HLA-G5. This in fact makes it possible to obtain the physiological form HLA-G5/ $\beta 2M$ .

10 **1. Construction of transfer vectors for recombination with the BacTen virus.**

**a- Insertion of the  $\beta 2M$  gene into a transfer vector:**

The  $\beta 2M$  coding DNA sequence is inserted into the BgIII-Kpn1 sites of the pTen12 transfer vector (Quantum). The  
15 recombinant clone is verified by enzymatic digestion and then amplified and sterilized for the purpose of cotransfection with the DNA of the linearized BacTen baculovirus.

20

**b- Insertion of the gene encoding HLA-G5 into a transfer vector:**

The sequence encoding the HLA-G5 molecule is inserted  
25 into the BgIII-Kpn1 sites of the pTen12 transfer vector. The recombinant clone is verified by enzymatic digestion and then amplified and sterilized for the purpose of cotransfection with the DNA of the linearized BacTen baculovirus (Quantum).

30

**2. Construction of recombinant baculoviruses**

The first step consists in producing, firstly, HLA-G5A recombinant baculoviruses and, secondly,  $\beta 2M$   
35 recombinant baculoviruses. The two transfer vectors carrying the  $\beta 2M$  and HLA-G5A genes are placed together with linear BacTen in order to cotransfect Sf9 insect cells in culture. The cotransfection supernatants are harvested and lysis plaque cloning is carried out in



order to isolate the recombinant baculovirus clones. In order to guarantee the quality of the construct, the DNA of the viral clones is extracted and the insertion of the gene into the correct viral *locus* is verified by PCR. Six clones were obtained for each of the constructs, and each one was used to reinfect Sf9 cells. The culture supernatants and also the cells where then recovered after centrifugation. This protein is purified by immunoaffinity using PAG5-6 antibodies (see Example 4). The structure of the protein is verified by western blot, both with PAG5-6 antibodies (anti-HLA-G5) and BIG6 antibodies (Immunotech) (anti- $\beta$ 2M) specific, respectively, for the soluble HLA-G5 form and for  $\beta$ 2M. One clone was selected from the 6 clones producing HLA-G5 and one clone was selected from the 4 clones producing  $\beta$ 2M. One HLA-G5 $\alpha$  clone and one  $\beta$ 2M clone were used to coinfect Sf9 cells. It is possible to show, by western blot analysis and immunoprecipitation with a conformational antibody W6/32 (IgG2a, against HLA class I  $\alpha$  chains associated with  $\beta$ 2-m (Sigma)), that the supernatant from this coinfection contains the HLA-G5 protein associated with  $\beta$ 2M; it is thus in a form close to the physiological form. These Sf9 cells therefore make it possible to obtain large amounts of soluble HLA-G5 protein.

**EXAMPLE 2 : Demonstration of the regulatory role of HLA-G in the T-dependent phenomena linked to psoriasis**

**1. Origin of the skin biopsies**

Skin biopsies exhibiting plaques typical of chronic psoriasis lesions, derived from patients who had undergone no local or general treatment during the 15 days preceding the day of the biopsy, were analyzed.

Healthy skin biopsies derived from patients who have undergone reductive breast surgery are used as a negative control.

The samples are separated into two, one portion is immediately frozen in liquid nitrogen for the RT-PCR analysis and the remaining portion is embedded in OCT (Miles Inc Diagnostics Division) for the immunohistochemical analysis.

## 2. Demonstration of all of the HLA-G transcripts and of the transcript specific for the soluble HLA-G5 isoform in the skin biopsies exhibiting psoriasis lesions

### a) Materials and methods

The messenger RNAs are extracted from frozen biopsies of 6 specimens of lesioned psoriatic skin (Pso1-Pso-6, figures 1 and 3) and of 4 specimens of normal skin (healthy skin 1-healthy skin 4, figures 2 and 3) under the following conditions: the samples are placed together with the RNA Now reagent (Biogentex, Inc.) and homogenized using an ultraturrax homogenizer (IKA Labortechnik), according to the manufacturer's recommendations. The quality of the extracted RNA is verified by electrophoresis on a 1.5% agarose gel under denaturing conditions. 5 µg of RNA are reverse transcribed into cDNA using an oligo dT primer 12 to 18 oligonucleotides long (Gibco BRL) and the Moloney virus (M-MLV) reverse transcriptase, for 1 h at 42°C. The cDNA obtained is then amplified by PCR with, firstly, primers which recognize all of the HLA-G transcripts [primer G.257 (exon 2) and 3G.U (untranslated 3' end)], used for the PCR amplification of the HLA-G transcripts corresponding to the various known isoforms of HLA-G (figures 1 and 2) and, secondly, primers specific for the soluble HLA-G5 sequence, namely primers G.526 and G.i4b (figure 3).

More precisely, the various primers and probes used are, consequently, as follows:

- G.526 (exon 3) and G3.U (3'UT) for the G1, G4 and G5 isoforms;
- G.526 (exon 3) and G.i4b (intron 4) for the G5  
5 isoform;
- G.-3 (partially covering exons 2 and 4) and G3.U (3'UT) for the G2 and G6 isoforms;
- 10 - G.3-4 (partially covering exons 2 and 5) and G3.U (3'UT) for the G3 isoform.

The PCR conditions are as follows: 1 minute at 90°C, 90 s at 65°C (61°C for the pair of primers G.526 and  
15 G.i4b) and 2 minutes at 72°C, for 35 cycles. The PCR products are separated by electrophoresis on a 1.5% agarose gel.

20  $\beta$ -actin transcripts are coamplified, for 16 cycles using specific primers (Clontech), in order to standardize the amount of RNA between the various samples.

25 The PCR products are then transferred onto a nylon membrane (Hybond N<sup>+</sup>, Amersham) and hybridized with a radiolabeled probe, and the intensity of the hybridization signal is quantified by densitometry.

The specific HLA-G probes are as follows:

- 30 - GR specific for exon 2, which recognizes all the HLA-G transcripts, and
- G.I4 F specific for intron 4, which only recognizes  
35 the HLA-G5 transcript.

The primers and probes above are described in P. MOREAU et al., C.R. Acad. Sci. Paris, Sciences de la vie/Life Sciences, 1995; 318; 837-42).

The cDNA from JEG-3 choriocarcinoma cells (ATCC), which have a high level of HLA-G transcription, is used as a positive control. In the assays above, the JEG-3 line is cultured in a DMEM medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum, antibiotics and 2 mM L-glutamine. The cell lines do not contain microplasmas.

The specific HLA-G bands are revealed by hybridization with the GR-specific probe located in exon 2. The PCR products, coamplified during the same reaction, with the  $\beta$ -actin primers are detected on the same membrane using a  $\beta$ -actin probe.

#### b) Results

The results are illustrated in figures 1 to 3.

The HLA-G transcripts are detected in all the samples of lesioned skin exhibiting psoriasis lesions and in only 2 of the 4 samples of healthy skin. With the exception of one sample (sample 3), only the transcripts corresponding to the HLA-G1 and HLA-G5 isoforms are detected in the samples of lesioned skin. In the lesioned skin biopsies from 6 patients suffering from psoriasis, a higher HLA-G transcriptional level exists, in particular with regard to the G1/G5 isoform ( $p < 0.05$ ; figures 1 and 3).

In these samples, the signal for the soluble HLA-G5 isoform is absent from the 4 specimens of normal skin (figure 3), but is found in 3 of the 6 individuals exhibiting psoriasis (figure 3).

The set of results shows that the level of the HLA-G1 and -G5 transcripts is higher in the skin biopsies exhibiting psoriasis lesions than in the samples of healthy skin, and that the HLA-G5 transcript is

expressed only in the skin biopsies exhibiting psoriasis lesions.

3. Demonstration of the HLA-G protein in the skin  
5 biopsies exhibiting psoriasis lesions

a) Materials and methods

10 a<sub>1</sub>) Immunohistochemistry

Cryostat sections were prepared from frozen samples and the sections were then dehydrated in acetone at -20°C for 20 min and dried in the open air.

15 The immunolabeling is carried out using the *Dako EnVision+System Peroxide (AEC)* kit (Dako), according to the manufacturer's instructions.

The antibodies used are as follows:

20

- 87G and O1G specific for HLA-G (HLA-G1 to -G5) and 16G1 specific for HLA-G5 (D. Geraghty, Fred Hutchinson Cancer Research Center, Seattle, USA),

25

- 4H84 specific for the isoforms of HLA-G in the denatured form (M. McMaster, San Francisco, USA) - anti-ILT2 which recognizes a KIR involved in binding to HLA-G (Navarro et al., Eur. J; Immunol., 1999, 29, 277-283),

30

- W6/32: anti-MHC class I antigen (Sigma)

- anti-CD3 (Sigma)

35

- anti-CD14 (Sigma)

- mouse IgG2a: control antibody (Sigma),

a<sub>2</sub>) Double labeling

The double labeling is carried out with the following pairs of antibodies:

- 5 - 87G and anti-CD68
- 87G and anti-CD11c (Dako)
- anti-ILT2 and anti-CD3.

10

The conditions used are as follows: after incubation with normal goat serum, the sections are incubated with the first antibody (87G or anti-ILT2) for 60 min, rinsed and then incubated with a Texas red-coupled goat anti-mouse IgG antibody. The sections are then rinsed and incubated for 30 min with the second antibody coupled to fluorescein isothiocyanate (FITC): anti-CD68 or anti-CD11c antibody when the first antibody is 87G, or anti-CD3 antibody when the first antibody is anti-ILT2.

20

In the control sections, the first antibody (87G or anti-ILT2) is replaced with the control antibody (mouse IgG2a).

25

The sections are then analyzed by laser confocal microscopy.

## **b) Results**

30

b<sub>1</sub>) Detection of all the HLA-G proteins and of the soluble HLA-G5 protein in the skin biopsies

No expression of the HLA-G protein is detected in the samples of healthy skin when using the 87G antibody or the 01G antibody which recognize all the isoforms of HLA-G.

35

On the other hand, HLA-G expression is detected in cells of the dermal papillae in the 9 samples of skin exhibiting psoriasis lesions. The level of HLA-G expression is variable depending on the samples; it is high in some samples. In addition, in only two samples, HLA-G expression is also detected in the epidermis, in areas close to the inflammatory cell infiltrate of the dermis, either in isolated loci of the inflammatory cells or in a few keratinocytes. The localization of HLA-G in these few keratinocytes is probably linked to the diffusion of the HLA-G5 isoform from the cells of the dermis expressing said isoform.

Specific labeling of the cells of the dermal papille is also detected when using the 16G1 antibody which specifically recognizes the soluble HLA-G5 isoform.

b<sub>2</sub>) Localization of the cells expressing HLA-G in the psoriasis lesions

Double labeling with the 87G antibody and the anti-CD3 antibody or the anti-CD14 antibody shows that HLA-G is colocalized in the cells expressing CD14; on the other hand, no colocalization of HLA-G with T lymphocytes (CD3<sup>+</sup>) is observed.

b<sub>3</sub>) The cells expressing HLA-G are CD68<sup>+</sup>, CD11c<sup>+</sup> macrophages

The labeling of serial sections of the samples of skin exhibiting psoriasis lesions, with the 87G antibody and the anti-CD68 or anti-CD11c antibodies which specifically recognize macrophages, demonstrates that virtually all of the cells which express HLA-G also express CD68 and CD11c and are therefore macrophages.

b<sub>4</sub>) The ILT2-type KIR receptor is present in the T lymphocytes infiltrating psoriasis lesions

The labeling of skin biopsies exhibiting psoriasis lesions, with the anti-ILT2 antibody, shows the presence of a dense infiltrate in the superficial dermis. On the other hand, no labeling is detected in the dermis of the samples of healthy skin. In addition, double labeling experiments with the anti-CD3 and anti-ILT2 antibodies show the presence of a few double-labeled cells in the dermal papillae.

In summary, the set of results presented above shows that:

- in healthy skin, the expression of the HLA-G RNAs is low or undetectable and the HLA-G protein is absent,
- in psoriasis lesions, there is increased expression of the RNAs and of the protein of the HLA-G1 and -G5 isoforms,
- in psoriasis lesions, the HLA-G expression is localized in macrophages, and
- in psoriasis lesions, the infiltrating T lymphocytes express a receptor for inhibiting cytotoxic functions which is recognized by HLA-G (ILT2 receptor).

These results show the presence, in psoriasis lesions, of both cells expressing HLA-G (macrophages of the dermal papillae) and infiltrating cells responsible for the psoriasis lesions (CD3<sup>+</sup> T lymphocytes), expressing a receptor for inhibiting cytotoxic functions which is recognized by HLA-G (ILT2 receptor).

Consequently, these results demonstrate the direct role of HLA-G, and in particular of the predominant membrane-bound isoform HLA-G1 and of the diffusible soluble isoform HLA-G5, in the local regulation of the T-dependent phenomena responsible for psoriasis.



**EXAMPLE 3: Role of the  $\alpha 1$  extracellular domain of HLA-G in the inhibition of immune functions, and application in the treatment of psoriasis**

5    **1. Role of the  $\alpha 1$  extracellular domain of HLA-G in the inhibition of the cytotoxic activity of NK cells and of T cells**

10    **a) NK cells**

The use of cells transfected with each of the HLA-G1, -G2, -G3 or -G4 isoforms as target cells opposite immunocompetent *natural killer* cells present in peripheral blood makes it possible to demonstrate that  
15    each of the isoforms is capable of inhibiting the cytotoxic activity of *natural killer* cells (fig. 4). These experiments were carried out on more than ten healthy voluntary donors and the significance of the inhibition exerted by each of the isoforms was  
20    validated with statistical tests (fig. 4).

**b) T cells**

Similar *in vitro* cytotoxicity assays carried out with  
25    the same target cells opposite MHC-restricted CD8+ T cells specific for an antigenic peptide also demonstrated that each of the HLA-G1, -G2, -G3 and -G4 isoforms significantly inhibits the cytotoxic activity of these T cells (fig. 5).

30    Based on the structure of the HLA-G3 isoform consisting only of the  $\alpha 1$  extracellular domain possessing all the inhibitory properties described above, a conclusion may be drawn regarding the functionality of this domain.  
35    This domain therefore contains the functional motif of HLA-G and may therefore be used as a pharmacological agent for the purpose of immunotolerance.

**2. Application in the treatment of psoriasis**

The direct role of HLA-G, and in particular of the predominant membrane-bound isoform HLA-G1 and of the diffusible soluble isoform HLA-G5, in the local  
5 regulation of the T-dependent phenomena responsible for psoriasis was demonstrated in example 2.

The role of HLA-G, and in particular of the  $\alpha 1$  domain of said isoforms, in the inhibition of T functions was  
10 demonstrated above.

Consequently, the set of results indicates that a pharmaceutical composition containing said HLA-G isoform, in particular in diffusible soluble form, such  
15 as the HLA-G5 isoform, has a protective role in the treatment of psoriasis.

**EXAMPLE 4: Production of an antibody, named PAG5-6, which specifically recognizes the soluble forms of HLA-G (HLA-G5 and HLA-G6), in the form of a polyclonal serum produced in rabbits.**  
20

The immunization of rabbits with an immunogenic peptide consisting of a 21 amino acid synthetic peptide  
25 corresponding to the C-terminal portion encoded by intron 4 of the soluble HLA-G forms, the sequence of which is SKEGDGGIMSVRESRSLSEDL (SEQ ID NO:1), coupled to the KLH carrier protein, makes it possible to produce a polyclonal serum which specifically  
30 recognizes the soluble forms of HLA-G (HLA-G5 and HLA-G6) via immunoprecipitation techniques, immunoprinting techniques (Western blot), immunohistochemistry techniques and immunoenzymatic techniques of the ELISA type. The serum is purified on an affinity  
35 column (protein G-sepharose) and may be used both for detecting, titrating and purifying the soluble HLA-G forms.

5

CLAIMS

1. The use of a composition essentially consisting of  
at least one soluble form of HLA-G and of at least  
5 one pharmaceutically acceptable vehicle, for  
preparing a medicinal product for treating  
inflammatory pathological skin conditions.
2. The use as claimed in claim 1, characterized in  
10 that said soluble form of HLA-G is selected from  
the group consisting of the soluble isoforms of  
HLA-G comprising at least the  $\alpha 1$  extracellular  
domain and the solubilized forms of HLA-G1, HLA-  
G2, HLA-G3 or HLA-G4.
3. The use as claimed in claim 1 or claim 2,  
15 characterized in that said composition comprises  
between 0.1 and 5  $\mu\text{g/ml}$ , preferably between 0.5  
and 2.5  $\mu\text{g/ml}$ , of soluble form of HLA-G.
4. A method for preparing a soluble HLA-G,  
20 characterized in that it comprises the following  
steps:
  - 25 - coinfecting insect cells with a baculovirus  
containing the  $\beta_2\text{M}$  cDNA and another baculovirus  
containing the  $\alpha$  chain of a soluble isoform of  
HLA-G;
  - 30 - culturing the transfected insect cells, and
  - harvesting the supernatants and purifying the  
soluble isoform of HLA-G expressed.
- 35 5. The method as claimed in claim 4, characterized in  
that said soluble isoform of HLA-G is purified  
using an antibody specific for the soluble  
isoforms of HLA-G.

6. The method as claimed in claim 5, characterized in that said antibody is obtained by immunizing nonhuman mammals, in particular rabbits, with an immunogenic peptide of SEQ ID NO:1 coupled to the KLH carrier protein.
- 5
7. A soluble anti-HLA-G antibody, characterized in that it is obtained by immunizing nonhuman mammals, in particular rabbits, with an immunogenic peptide consisting of a 21 amino acid synthetic peptide of SEQ ID No:1 coupled to the KLH carrier protein.
- 10

-24-

### Abstract

The invention concerns a composition consisting essentially of at least a soluble form of HLA-G and at least an acceptable pharmaceutical carrier for preparing a medicine for treating inflammatory pathological conditions of the skin. The invention also concerns a method for obtaining soluble forms of HLA-G and antibodies directed against said soluble forms.

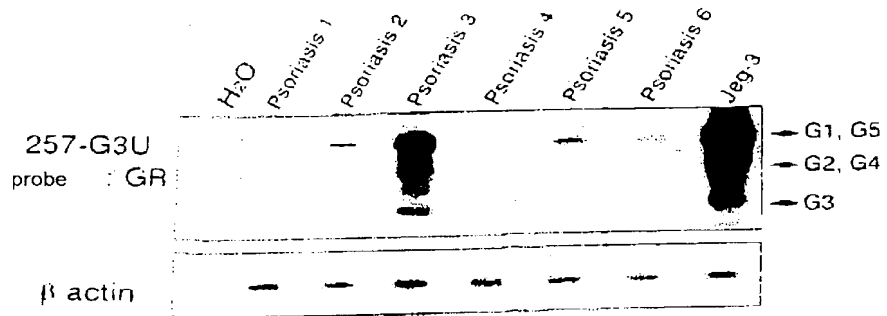


FIGURE 1

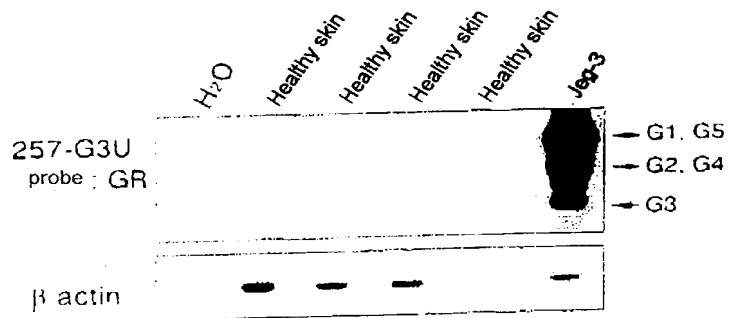


FIGURE 2

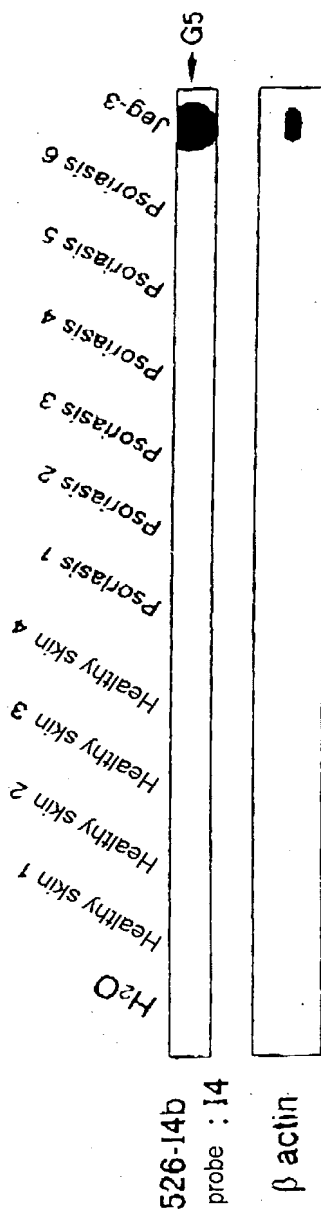


FIGURE 3



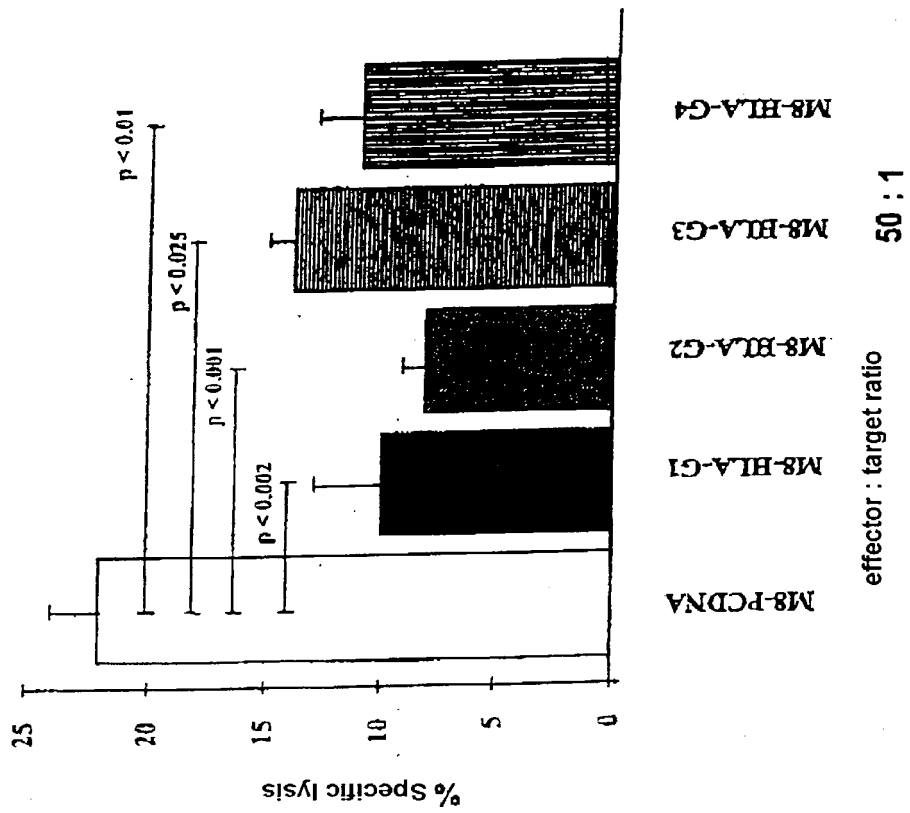


FIGURE 4

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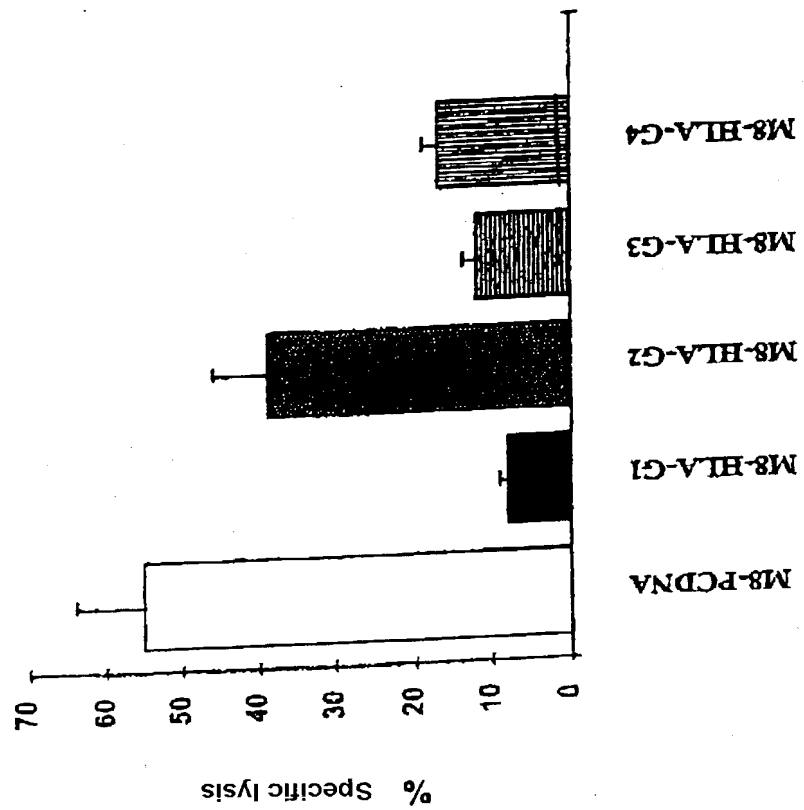


FIGURE 5

**Declaration and Power of Attorney for Patent Application**  
**Déclaration et Pouvoirs pour Demande de Brevet**  
**French Language Declaration**

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

☐ ci-joint

☐ a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**COMPOSITIONS CONTAINING SOLUBLE FORMS OF HLA-G FOR TREATING INFLAMMATORY SKIN PATHOLOGIES**

the specification of which :

☐ is attached hereto.

☒ was filed on December 18, 2001

as United States Application Number or PCT International Application Number No. 09/926,778

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

### French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

Prior Foreign application(s)  
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Country)  
(Numéro) (Pays)

99/07736 FRANCE

(Number) (Country)  
(Numéro) (Pays)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

(Application No.) (Filing Date)  
(N° de demande) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365(d) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

(Application No.) (Filing Date)  
(N° de demande) (Date de dépôt)

(Application No.) (Filing Date)  
(N° de demande) (Date de dépôt)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority claimed  
Droit de priorité  
revendiqué

(Day/Month/Year Filed) ☒ Yes ☐ No  
(Jour/Mois/Année de dépôt) Oui Non

18/06/1999  
(Day/Month/Year Filed) ☐ Yes ☐ No  
(Jour/Mois/Année de dépôt) Oui Non

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Filing Date)  
(N° de demande) (Date de dépôt)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Status) (patented, pending, abandoned)  
(Statut) (breveté, en cours d'examen, abandonné)

(Status) (patented, pending, abandoned)  
(Statut) (breveté, en cours d'examen, abandonné)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**French Language Declaration**

**POUVOIRS :** En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques : (mentionner le nom et le numéro d'enregistrement).

**POWER OF ATTORNEY :** As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all business in the Patent and Trademark Office connected therewith : (list name and number)

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|  |   |
|--|---|
| 1-00<br>Nom complet de l'unique ou premier inventeur<br><b>ARACTINGI/Selim</b>         | Full name of sole or first inventor<br><b>ARACTINGI/Selim</b> |
| Signature de l'inventeur <i>[Signature]</i> Date <b>6/3/2002</b>                       | Inventor's signature <i>[Signature]</i> Date                  |
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| Nom complet du second co-inventeur, le cas échéant<br><b>CAROSELLA/Edgardo Delfino</b> | Full name of second joint inventor, if any                    |
| 2-00<br>Signature de l'inventeur <i>[Signature]</i> Date <b>18.11.2001</b>             | Second inventor's signature Date                              |
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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

## French Language Declaration

|     |  |   |
|-----|--|---|
| 300 | Nom complet du troisième co-inventeur, le cas échéant<br><b>DAUSSET/Jean</b>         | Full name of third joint inventor, if any                             |
|     | Signature de l'inventeur <i>[Signature]</i> Date <i>20-03-02</i>                     | Third inventor's signature <i>[Signature]</i> Date                    |
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| 400 | Nom complet du quatrième co-inventeur, le cas échéant<br><b>KHALIL/DAHER/Iman</b>    | Full name of fourth joint inventor, if any                            |
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|     | Nationalité<br>Française   | Citizenship   |
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| 500 | Nom complet du cinquième co-inventeur, le cas échéant<br><b>MOREAU/Philippe</b>      | Full name of fifth joint inventor, if any                             |
|     | Signature de l'inventeur <i>[Signature]</i> Date <i>13/03/02</i>                     | Fifth inventor's signature <i>[Signature]</i> Date <i>13/03/02</i>    |
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|     | Nationalité<br>Française   | Citizenship   |
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| 600 | Nom complet du sixième co-inventeur, le cas échéant<br><b>PAUL/Pascale</b>           | Full name of sixth joint inventor, if any                             |
|     | Signature de l'inventeur <i>[Signature]</i> Date <i>21/02/02</i>                     | Sixth inventor's signature <i>[Signature]</i> Date <i>21/02/02</i>    |
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#7

SEQUENCE LISTING

<110> ARACTINGI, SELIM  
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DAUSSET, JEAN  
KHALIL DAHER, IMAN  
MOREAU, PHILIPPE  
PAUL, PASCALE  
ROUAS-FREISS, NATHALIE

<120> COMPOSITIONS CONTAINING SOLUBLE FORMS OF HLA-G IN THE TREATMENT OF  
INFLAMMATORY SKIN PATHOLOGIES

<130> 217365US0PCT

<140> US 09/926,778

<141> 2001-12-18

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